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PREVALENCE OF SUBCLINICAL MASTITIS IN BUFFALOES (BUBALUS BUBALUS), IN CHHATTISGARH, INDIA

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ABSTRACT

A study was carried out from February 2013 to January 2015 in 1650, buffaloes of organized and unorganized farms of Durg, Rajnandgaon, Raipur and Bilaspur districts of Chhattisgrah State in India, to determine the prevalence of subclinical mastitis. Milk samples were collected to detect subclinical mastitis in individual animal based on milk somatic cell count (SCC) and MCMT. The overall prevalence of buffalo subclinical mastitis accounted for 41.51% (685/1650). Prevalence of SCC was evaluated on the basis of geographical distribution, parity, age and stage of lactation, pattern of milking etc. More cases of SCC were observed in unorganized farms with Prevalence ranges from38.41% to 44.93% in Durg and Rajnandgaon, respectively. Buffaloes of 9-11 year suffered more that is 57.51% as compared to younger with early lactation (52.78%). More number of cases was observed in rainy season and least in dry climate. Prevalence % of MCMT score + was observed more with SCC range from 2.12-2.85×10^5 cells/ml of milk.

KEYWORDS: Buffaloes, Subclinical, Mastitis, Prevalence & Milk

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Original Article

INTRODUCTION

Subclinical mastitis (SCC) remains the most economically deteriorating and zoonotic potential disease for dairy industry and consumers worldwide, irrespective of species of animal (Ojo et al., 2009). SCC has, in principal, been considered a problem in high producing cattle in developed countries, but through field investigations and improved recordings it is now recognized as a significant problem even in low yielding animals in developing countries, with a detrimental effect on animal production and public health (Tesfaye et al., 2010). Previous studies conducted in different countries indicate the distribution and economic importance of the disease.

In Nepal, according to Ng et al5 largest proportion of losses in milk, results from decreased milk production i.e., Rs.4287 or USD 63 per buffalo per lactation In India, Dua (2001) has reported annual losses, due to mastitis to the tune of Rs 60.5321 billion of which, Rs. 43.6532 billion has been attributed to sub-clinical mastitis. Buffaloes are the main dairy animal worldwide in developing countries. The prevalence of bovine subclinical mastitis was reported 30-40 times more than clinical mastitis cattle (Bhanderi and Garg, 2012). The

inflammatory reactions like swelling, heat, pain and indurations of udder are commonly attributed with clinical mastitis but in subclinical mastitis no apparent visible changes are seen in udder but severe drop in milk production and significant changes in milk composition are recorded (Radostitis *et al*., 2010). The lower quality and the diminished sustainability of milk with a high SCC both constitute a potential health risk and also affect the possibility of producing other dairy products(Andersson et al. 2011). The need to detect the udder infection Zoonotic diseases which is potentially transmitted by raw cow milk, include brucellosis, caseous lymphadenitis, leptospirosis, listeriosis, melioidosis, Q-Fever, Staphylococcal food poisoning, toxoplasmosis and tuberculosis (Radostits et al., 2007). Therefore, this study aimed to evaluate the prevalence of subclinical mastitis (SCM), in apparently healthy animals in an organized and unorganized farm and the associated risk factors.

MATERIALS AND METHODS

Prevalence of subclinical mastitis over a period of two years from February 2013 to January 2015 in buffaloes in organized and unorganized dairy farms in and around Durg, Rajnandgaon, Raipur and Bilaspur districts of Chhattisgarh state was made, on the basis of history of sudden drop in milk yield and confirmed diagnosis was made by MCMT, somatic cell count

Collection of Milk Samples

About 50-60 ml of milk sample was collected from apparently healthy buffaloes of Murrah breed after proper disinfection of teat surface with 70% ethanol and discarding few strips of fore-milk. Indirect tests like MCMT (Modified California mastitis test) and SCC were performed on spot at dairy farms.

Modified California Mastitis Test (MCMT)

MCMT was conducted during milking of cows as per guidelines of Devi (1997). A plastic paddle with four chambers or shallow cups was used to perform the test. About 5 ml of milk was directly striped in to labeled cups viz. LF, LH, RF and RH for the respective four quarters with equal quantity of the test reagent (3% sodium lauryl sulphate solution. The mixture of milk and reagent was shaken gently in a rotating manner of the paddle in a horizontal plane. Immediately after mixing, the reaction was graded by intensity of gel formation as described in Table 1.

Table 1: MCMT Grade in Milk in SCC Affected Buffaloes

MCMT- Grade	Description	Score point
N (Negative)	No change	0
T (Trace)	Slime formed which disappeared with continuous movement of paddle	1
+ (Weak)	Distinct slime, but no gel formation	2
++ (Distinct positive)	Viscous with gel formation, adhered to the margin	3
+++ (Strong positive)	Gel formation with convex projection which did not dislodge after swirling movement of the paddle	4 and 5

Somatic Cell Count (SCC)

The gold standard is to measure inflammation through cytological investigation (Pyrola, 2003), i.e., counting somatic cells. In SCM the most significant abnormality of the milk is the increase in SCC. A total of SCC > 200000 cell/ml of milk is an indicator, for intra mammary infection in case of lactating dairy crossbred cows (Singh and Ludri, 2001). Somatic cell count was performed using the instrument the Porta SCC milk test, procured from the Porta Check,

Moorestown, New Jersey, U.S.A.

RESULTS AND DISCUSSIONS

Prevalence of subclinical mastitis and its relation with age, number of lactation, stage of lactation, quarter-wise distribution, pattern of milking and seasonal incidence in buffaloes of Chhattisgarh was studied by screening the animals on the result of indirect tests like MCMT and SCC. The prevalence of subclinical mastitis was correlated with age, stage of lactation, quarter wise distribution, pattern of milking and seasonal incidence respectively.

Area Wise Prevalence

Present study (Table 2) revealed that, the SCM prevalence of 41.51% obtained in this study is lower than the prevalence of 51.8 %, obtained by Iragua et al., (2015). Ali et al., (2011) also observed higher prevalence of 44% in Buffaloes of Punjab. Further analyses revealed that on an average 27.5 % of the samples showed SCM in organized farms as compared to 76% in unorganized farms. This difference in the observed prevalence of mastitis among studies maybe attributed to various factors like management, environmental, animal risk factors and causative agents (Radostits*et al*., 2007). On the basis of geographic allocation, Rajnandgaon district was affected least as compared to other studied areas due to more number of organized farms in this district.

Table 2: Prevalence of Bubaline Subclinical Mastitis in and Around Durg, Rajnandgaon, Raipur and Bilaspur Districts of Chhattisgarh State

District	Total No. of Buffaloes Examined	No. of Buffaloes Examined in Organized Dairy Farm	No. of Buffaloes Examined in Un-Organized Dairy Farm	No. of Positive Buffaloes	Prevalence Percentage
Durg	296	180(54)	116(79)	133	44.93
Rajnandgaon	604	466(111)	138(121)	232	38.41
Raipur	412	304(89)	108(79)	168	40.77
Bilaspur	338	224(69)	114(83)	152	44.79
Total	1650	1174(323)	476(362)	685	41.51

(Values in parenthesis denotes number of positive cases)

Age Wise Prevalence of Subclinical Mastitis

The prevalence of subclinical mastitis in different age groups of lactating buffaloes was studied during the study period. Animals of the age group 3 years and above were included in this study (Table 3).

Table 3: Prevalence of Bubaline Subclinical Mastitis among Different Age Groups in and around Durg, Rajnandgaon, Raipur and Bilaspur Districts of Chhattisgarh State

Age Group (years)	No. of Buffaloes Examined	No. of Positive Buffaloes	Prevalence Percentage Significant (P<0.05)
3-5	385	130	33.76
6-8	585	268	50.81*
9-11	545	257	57.51
12-14	95	25	43.31

Increasing age increased the risk of mastitis. There was a significant difference in prevalence between animals of different age categories (P<0.05). Prevalence followed increasing trend and highest prevalence (57.51%) was found in lactating Buffaloes of age 9-11 years, thereafter decreased and 41.31% was observed in animals of 12-14 years of age(Table 3). The reason of lowest prevalence in animals of 14years and above is due to their decreased milk yield. Older

cows are more susceptible to SCM (Sharma et al.,2012), because of the breakdown of the streak canal barriers and udder tissue with progressive ageing process (Schalm et al.,1971). Several studies agreed with the present findings of higher prevalence of subclinical mastitis with the advancing parity (Srinivasan et al., 2013,)

Table 4: Prevalence of Subclinical Mastitis in different Stages of Lactation in Buffaloes in and around Durg, Rajnandgaon, Raipur and Bilaspur Districts of Chhattisgarh State

Different Stages of Lactation	No. of Buffaloes Examined	No. of Positive Buffaloes	Prevalence Percentage
Early	610	322	52.78
Mid	595	221	37.14
Late	445	142	31.91

Table 4 presented the prevalence percentage of SCM on the basis of lactation period. Results of the present study revealed higher prevalence of SCM in early lactation (52.78%) and lowest in late lactation (31.91), that are comparable with the findings of Khanal and Pandit, (2013). Our result is in contrary to this Srinivasan et al., (2013) observed higher prevalence in late lactation period.

During early stage of lactation, the production stress on udder increases pressure within the udder that causes broadening of teat canal and teat sphincter and that might be risk for intra-mammary infection (Guarín and Ruegg, 2016). The neutrophil and lymphocyte functions are diminished, during the peri-parturient period. Nutritional, hormonal and metabolic alterations are associated with pregnancy and immune system is compromised around calving (Radostits et al., 2007)

In early stage of lactation high yielding animals are in negative energy balance during early stage of lactation and increased level of non esterifies fatty acids (NEFA) and β- hydroxy butyric acid (BHB) impairs the migration and phagocytic activity, enhancing thesusceptibility of mammary gland to mastitis pathogens (Suriyasathaporn et al., 2000)

Table 5: Distribution of Single and Multiple Quarter Involvement in Subclinical Mastitis in Buffaloes

Animal	No. of Animals	No. of Functional Quarters	Quarter Wise Distribution			
			LF	LH	RF	RH
No. of buffaloes examined	1650	6460(140)	1631	1620	1621	1588
No. of buffaloes found positive	685	2675 (65)	629	720	593	732
Percentage	41.51	41.40	38.56	44.44	36.50	46.12

(Values in parenthesis include non functional teats)

Present research showed that prevalence rate was found highest in right hind which was followed left hind, left fore and right for (Table 5). This result was quite contrasting to the result obtained by Kanhal and Pandit, 2013, who found the incidence to be highest in left hindquarters (34.2%), followed by right hind quarters (31.6%). These findings are in concordance with those reported by Bansal *et al.* (2004) and Viswakarma (2008). However, the result is quite contrasting to the result obtained by Kanhal and Pandit, 2013. The higher prevalence in hind quarters may be due their closeness with dung and urine, so they are more prone to infection. In buffaloes, more milk yield from hind quarters also make them more susceptible to infection (Srinivasan et al., 2013).

Table 6: Prevalence of Subclinical Mastitis in Buffaloes on the Basis of Seasonal Variation in and Around Durg, Rajnandgaon, Raipur and Bilaspur Districts of Chhattisgarh State

Season	Months	Year 2013-14	Seasonal Total	Year 2014-15	Seasonal Total	No. of Cows Positive	Prevalence Percentage
Winter (October-January)	October	17	59	14	46	189	27.59
	November	14		11			
	December	16		9			
	January	12		12			
Summer (February-May)	February	41	136	34	122	115	16.78
	March	39		37			
	April	31		22			
	May	25		29			
Rainy (June-September)	June	18	61	16	69	381	55.62
	July	14		18			
	August	16		14			
	September	13		21			

The prevalence of a pathogen is influenced by parity, type of sample and season (Hagnestan*et al*., 2009). Present study revealed higher prevalence (55.62%) of mastitis during rainy months followed by winter and least in summer season (Table 6). These findings are in consonance with the findings of Dhakal et al. (2007), who reported an incidence of bovine mastitis around 37.3%during rainy season. The possible reason for increase incidences of mastitis cases during the rainy season is humid weather, higher calving rate and peak lactation. Due to high lactation, the immunity of mammary glands decreases and become highly susceptible for bacterial pathogens (Kaur et al., 2015). In contrary to our findings, Salsberg*et al.* (1984) observed that, somatic cell counts increased more during summer months, from June to August in Holstein cows than in cooler months.

Table 7: Prevalence of Subclinical Mastitis in Buffaloes on the Basis of Pattern of Milking in Cows in Durg, Rajnandgaon, Raipur and Bilaspur District of Chhattisgarh

Pattern of Milking	Positive Cases (total=685) (Values in Parenthesis include Positive Cases)
MACHINE	94 (13.72)
FULL HAND	183 (26.71)
FISTING	213 (31.09)
KUCKLING	195 (28.46

Manual milking methods adopted mainly in unorganized farms included in this study was the major predisposing factors to increase the prevalence of SCC (Table 7). Milkers often do not disinfect their hands and teats during and between milking of different cows, Milking of mastitic animals prior to the healthy ones might have important factor for increased prevalence of subclinical mastitis. (Radostits*et al.*, 2007). In addition incomplete emptying of milk from udder by manual milking could have led into trauma of the udder and provide good culture media for the growth of microorganism hence susceptible for mastitis (Shem et al., 2002).

Table 8: Correlation of MCMT Score with SCC Range in Buffaloes Affected with SCM

MCMT Score	Prevalence Percentage	SCC Range ($x10^5$ Cells/ml)
Trace	11.46	1.52 - 1.98
+	44.36	2.12 - 2.85
++	28.14	3.11 - 3.34
+++	16.04	3.55 - 3.98

Present study revealed that out of total 2740 quarters the number of functional were 2675 and the milk samples of buffaloes subjected to MCMT, the maximum cases belonged to grade + followed by ++, +++ and trace respectively (Table 8). During the course of study, it was found that SCC of milk in buffaloes ranged from 1.52 to 3.98 $x10^5$ cells per ml milk respectively. Prevalence percentage of 2.12 - 2.85 $x10^5$ cells per ml milk range was appeared highest. The present findings corroborate with the reports of Jones and Bailey (2009), who recorded a range of SCC which is below 2.0 X 10^5 cells/ml in healthy cows. However, Syed *et al*. (2009) have reported normal SCC range below 2.50 X 10^5 cells/ml, while Radostits *et al*., (2007) reported 1.0 X 10^5 cells/ml as the normal range of SCC in the milk, from healthy individual quarters.

CONCLUSIONS

SCM associated several risk factors including age, stage of lactation, milk production, farming system and hygiene measures so prevalence of SCM is more in unorganized farm. The highest prevalence of SCM was found in old age and late lactating animals. Good management practices might involve reduction of prevalence of subclinical mastitis.

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